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Stroke volume and sympathetic responses to lower-body negative pressure reveal new insight into circulatory shock in humans☆

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Abstract

We measured various hemodynamic responses and muscle sympathetic nerve activity (MSNA) in human subjects during a graded lower body negative pressure (LBNP) protocol to test the hypotheses that: (1) reduced stroke volume (SV) is linearly related to increased MSNA; and (2) the onset of symptoms of impending cardiovascular collapse is associated with hypoadrenergic responses to central hypovolemia. We measured heart rates, arterial blood pressures, sympathetic neural activity (MSNA; peroneal nerve microneurography), and relative changes (% Δ) in SV (thoracic electrical bioimpedance) in 13 men during exposure to graded levels of LBNP. After a 12 min baseline data collection period, LBNP was initiated at − 15 mm Hg for 12 min followed by continuous stepwise increments to − 30, − 45, and − 60 mm Hg for 12 min each. Eight subjects completed the LBNP protocol (finishers), while the protocol was terminated prematurely during − 60 mm Hg in five subjects due to onset of symptoms of cardiovascular collapse (nonfinishers). Of these subjects, we were able to record MSNA successfully throughout the LBNP protocol in four finishers and two nonfinishers. The relationship between average change in stroke volume and average change in MSNA was linear (% ΔMSNA 464 3.6 [% ΔSV], r^2 0.98). On average, MSNA was greater in the nonfinishers at each level of LBNP compared to finishers, but peripheral resistance was lower. Our results support the hypothesis that MSNA activation is *inversely related and linear* to stroke volume reductions during central hypovolemia. Sympathetic withdrawal rather than hypoadrenergic function may represent a fundamental mechanism for the development of circulatory shock.
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1. Introduction

Individuals who survive circulatory shock possess an enhanced physiologic reserve capacity to deal with drastic hemodynamic fluctuations, but such protective mechanisms have not been clearly defined. The primary problem is that circulatory shock has historically been identified by the appearance of systemic hypotension and accompanying symptoms such as pallor, restlessness, and general weakness (among others) (Orlinsky et al., 2001), but these symptoms are consequences, not mechanisms. Physiologic adjustments occurring *before* the onset of symptoms must be identified in an effort to understand why certain individuals survive circulatory shock. The application of such knowledge could prove critical to the development of biomedical devices and/or therapeutic methods to save lives.

Insufficient organ perfusion consequent to inadequate oxygen delivery heralds the onset of hypotension (Orlinsky et al., 2001) and is associated with increased mortality due to organ failure (Shoemaker et al., 1992). Therefore, maintenance of adequate oxygen delivery is fundamental to surviving or avoiding circulatory shock (Bland et al., 1978; Boyd et al., 1996). During acute central hypovolemia, arterial pressure is maintained through sympathetic activation, resulting in systemic vasoconstriction and cardiac acceleration. Under these conditions, if cardiac output falls to critically low levels (i.e., about one-half of the resting value), sympathetic drive may withdraw suddenly, resulting

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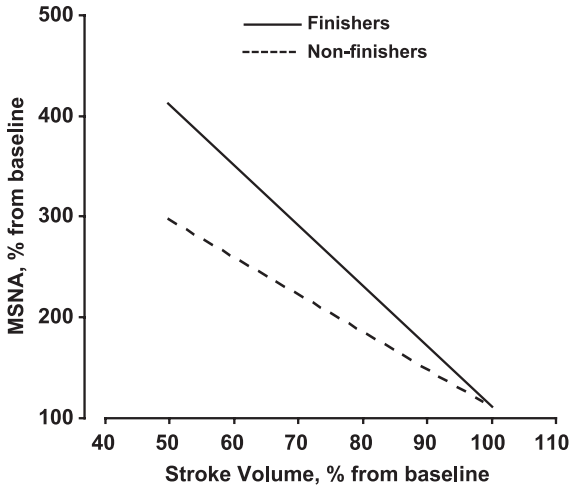

Fig. 1. The speculation that nonfinishers possess relatively decreased sympathetic reserve capacity (dashed line) during progressive central hypovolemia relative to finishers (solid line) is shown.

in circulatory collapse (Evans et al., 2001). Survivors of circulatory shock possess greater capacity to defend cardiac output during cardiovascular challenges, and this capacity may be related, in part, to a relatively greater neuroadrenal stress response (Orlinsky et al., 2001).

Neural traffic in postganglionic muscle sympathetic fibers (which may be recorded directly with the microneurography technique) increases with decreases in arterial pressure (Wallin and Nerhed, 1982), and released norepinephrine (NE) restores arterial pressure through constriction of vascular smooth muscle. Fig. 1 graphically depicts the concept that hypoadrenergic function during central hypovolemia represents a key mechanism underlying the ultimate progression to circulatory shock: to date, however, sympathetic microneurography has not been applied in an effort to explain autonomic mechanisms that may classify survivors and nonsurvivors. In the present investigation, we studied stimulus (stroke volume reductions)–response (peripheral efferent sympathetic traffic) relations during graded lower-body negative pressure (LBNP) to test the hypotheses that: (1) elevations of MSNA during central hypovolemia are proportional (i.e., linear) with reductions in stroke volume, and (2) the stroke volume–MSNA relationship will have less slope (i.e., less sympathetic reserve capacity) in subjects who develop symptoms of circulatory collapse during progressive LBNP.

2. Materials and methods

2.1. Subjects

Fourteen healthy men with a mean ($\pm$S.E.) age of 40 ± 3 years, height of 177 ± 2 cm, and weight of 80.2 ± 2.7 kg volunteered to participate as subjects for this investigation after all procedures and risks associated with the experiments were explained and their voluntary written informed consent to participate in the study was obtained. All procedures were reviewed and approved by the US Army Institute of Surgical Research Human Use Committee. All subjects were nonsmokers and normotensive, and their selection into the study was based on results of a detailed medical history to assure absence of cardiovascular disease. Individuals taking prescription drugs were excluded and subjects were refrained from taking any medications at the time of the experiments. Because of potential effects on vascular volume and autonomic functions, subjects were asked to refrain from exercise and stimulants such as caffeine and other nonprescription drugs 48 h prior to testing.

2.2. Experimental design

Each subject reported to the laboratory on one occasion and underwent a 60-min LBNP protocol consisting of a 12-min baseline period followed by exposure to -15, -30, -45, and -60 mm Hg decompression for 12 min each. LBNP was used as a method to induce central hypovolemia and subsequent hemodynamic and muscle sympathetic nerve activity (MSNA) responses similar to those measured during a *steady state* hemorrhage (Convertino, 2001; Cooke et al., 2004; Levine et al., 1994). The initial 2 min of each 12-min data collection period was used to allow the subject to reach a steady-state status *without data collection* (Levine et al., 1994). The next 5 min were required to collect our hemodynamic and MSNA data. The final 5 min were required to collect eye oximeter data that will be reported at a later date. Measurements during baseline and each LBNP level included heart rate (HR), stroke volume, arterial blood pressure, and MSNA. Because large, deep breaths (as might occur during a sigh) could confound the interpretation of the effects of LBNP on MSNA and interfere with thoracic impedance measurements, subjects breathed in time to a metronome set at a pace of 15 breaths/min, and did not deviate from this controlled breathing frequency during the period of data collection. Subjects were instructed not to contract their leg muscles during LBNP. Premature test termination was based on occurrence of any one or a combination of the following: (1) onset of symptoms of cardiovascular collapse such as a fall in systolic blood pressure (SBP) greater than 15 mm Hg and/or a fall in heart rate greater than 15 bpm (beats per minute) between adjacent 1-min measurements; (2) progressive fall in systolic blood pressure below 80 mm Hg; and (3) subject request due to symptoms such as nausea or dizziness. To assure subject safety, an ACLS-certified physician was present in the laboratory building during all LBNP tests.

2.3. Heart rate and blood pressure

Continuous HR was measured with a Hewlett-Packard monitoring system from a standard electrocardiogram

(ECG). A Finapres® fingercuff blood pressure monitoring device (Ohmeda, Englewood, CO) was placed at heart level to provide a noninvasive measurement of beat-by-beat SBP and diastolic blood pressure (DBP). The ability to obtain Finapres® blood pressure recordings was not affected by high levels (i.e., >45 mm Hg) of LBNP. Periodic blood pressure measurements were also conducted with a Colin automated sphygmomanometer to verify the readings obtained from the Finapres®. Mean arterial pressure (MAP) was calculated from results of the Colin sphygmomanometer (and automatically by computer for each beat-by-beat measurement) by dividing the sum of SBP and twice DBP by three.

2.4. Measurement of stroke volume

Stroke volume was measured noninvasively using thoracic electrical bioimpedance (TEB) with a Minnesota Impedance Cardiograph. The R-wave of the ECG was taken as a landmark to average d*Z*/d*t* waveforms over 10 cardiac cycles that were recorded at the beginning of minutes 2, 8, and 10 of each baseline and LBNP level. Stroke volume for minutes 2, 8, and 10 were determined as the average stroke volume from the 10 cardiac cycles, and average stroke volume at baseline and each level of LBNP was calculated as the average of the stroke volumes at 2, 8, and 10 min. Cardiac output was calculated as the product of heart rate and stroke volume, and systemic peripheral resistance (SPR) was calculated by dividing MAP by cardiac output.

2.5. Muscle sympathetic nerve activity

MSNA was measured directly with a Nerve Traffic Analyzer (Model 662C-1; University of Iowa Bioengineering, Iowa City, IA) according to the procedures described by Cooke (2000). Multifiber efferent sympathetic nerve traffic from peroneal nerve muscle fascicles at the popliteal fossa was recorded with tungsten microelectrodes (Frederick Haer and Co., Bowdoninham, ME). The course of the nerve was mapped by stimulating the nerve through the skin with a pencil-shaped electrode (10–50 V; 0.1 ms duration). The nerve was located when electrical stimulation produced muscle twitching in the lower leg. Once the nerve was located, two sterile wire electrodes (diameter approximately 0.2 mm) were introduced through the skin to a depth of approximately 0.5–1 cm; one electrode served as the ground electrode and the other as the recording electrode. The recording electrode was connected to a stimulator that delivered weak electrical pulses (1–5 V; 0.02 ms duration) through the electrode into the region of the nerve. The electrode was maneuvered into the nerve

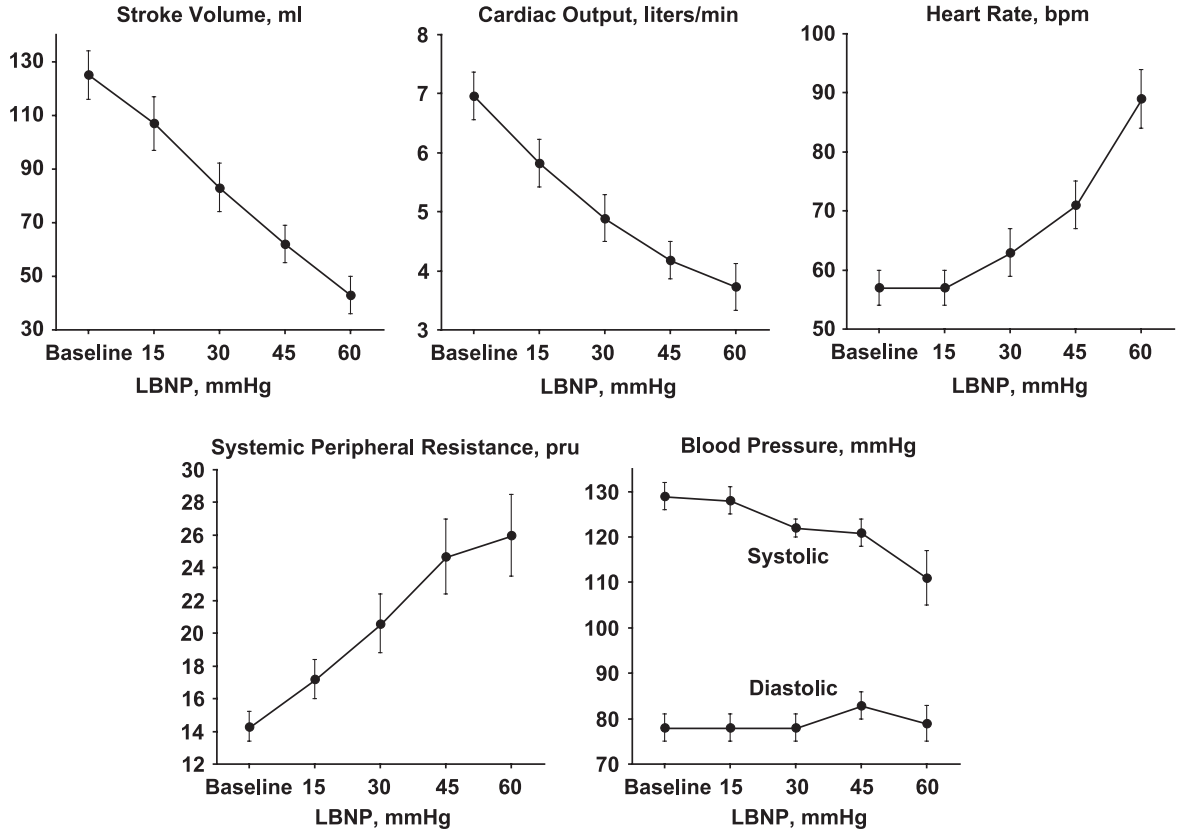

Fig. 2. Hemodynamic effects of graded LBNP. bpm beats per minute; pru peripheral resistance units (n 13).

eliciting muscular twitches, adjusted so that spontaneous sympathetic bursts were apparent, and the subsequent nerve activity was recorded. Both electrodes were connected to a differential preamplifier and then to an amplifier (total gain 70,000) where the nerve signal was bandpass-filtered (700–2000 Hz) and integrated (time constant 0.1 s) to obtain mean voltage neurograms. Satisfactory recordings of MSNA were defined by spontaneous, pulse synchronous bursts that increased during end-expiratory apnea, and did not change during tactile or auditory stimulation (which would indicate activity from skin). Bursts of MSNA were automatically detected through several criteria. Potential bursts were identified within a 0.5-s search window centered on an expected burst peak latency from preceding R-waves of 1.3 s. Potential bursts were also evaluated based on amplitude, using a signal-to-noise baseline criterion of approximately 3:1. Mean burst peak latency was calculated from the first iteration of the burst detection algorithm; these mean latencies were used as criteria for identifying bursts for a second iteration. This procedure was used until further latency corrections failed to result in an increase in the number of bursts detected, or in a change in average burst latency. MSNA was expressed as bursts per minute.

2.6. Statistical analysis

Standard descriptive statistics were performed on each of the response variables of interest with results presented as mean ± S.E. We described associations among stroke volume–MSNA changes and MSNA–peripheral resistance changes with linear regression analysis. Observations separating LBNP finishers from nonfinishers are unique but limited. For this reason, we present these data with descriptive, rather than inferential, statistics.

3. Results

Data from one subject were not analyzable and so we report hemodynamic results from 13 subjects. LBNP induced typical hemodynamic responses (Fig. 2). LBNP reduced stroke volume from an average of 125 ± 9 ml at baseline to 43 ± 7 ml at 60 mm Hg LNBP. Cardiac output was also reduced despite a reflex-mediated elevation in average heart rate from 57 ± 3 bpm at baseline to 89 ± 5 bpm at 60 mm Hg LNBP. LBNP increased systemic vascular resistance from an average of 14.3 ± 0.9 pru (peripheral resistance units) at baseline to 24.7 ± 2.3 pru at 45 mm Hg LNBP. The LBNP protocol was terminated on five subjects due to the onset of symptoms of cardiovascular collapse at − 60 mm Hg. These subjects were designated as "nonfinishers." Although these five subjects presented typical hemodynamic responses and subjective symptoms of cardiovascular collapse, no subjects lost consciousness.

Of the initial 14 subjects recruited to participate in this investigation, we were successful in recording MSNA from 10 subjects. In four subjects, successful placement and recording of MSNA was lost during LBNP of 15

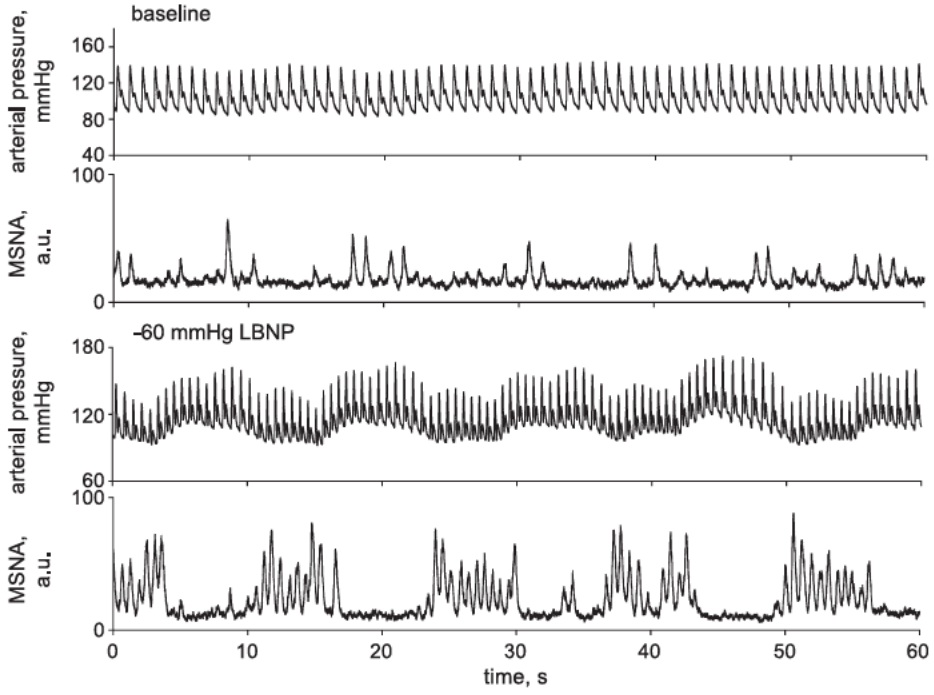

Fig. 3. Representative arterial pressure and MSNA during baseline, supine, and 60 mm Hg LBNP. au arbitrary units.

($n=1$) and 30 ($n=3$) mm Hg. We were able to record and analyze MSNA at baseline, 15, 30, and 45 mm Hg LBNP in six subjects, four of whom provided MSNA data at 60 mm Hg LBNP. Two of the six subjects with successful MSNA recordings through 45 mm Hg LBNP did not finish LBNP exposure at 60 mm Hg. In one of these subjects, we reported a complete withdrawal of MSNA at the time of cardiovascular collapse despite adequately high MSNA at LBNP levels prior to 60 mm Hg (Cooke and Convertino, 2002). Fig. 3 shows an original tracing from one subject depicting increases of arterial pressure instability at 60 mm Hg LBNP, and compensatory increases of MSNA.

The relationship between the average reduction in stroke volume and the average increase in MSNA was linear (% ΔMSNA = 464 – 3.6 [% ΔSV], $r^2 = 0.98$; Fig. 4A), as was the increase in peripheral resistance as a function of increases in MSNA (% ΔTPR = 464 – 3.6 [% ΔMSNA], $r^2 = 0.83$; Fig. 4B). MSNA increased from 13.7 ± 3.6 bursts/min at baseline to 35.9 ± 4.6 bursts/min at 60 mm Hg LBNP. The average MSNA response to graded LBNP in the two nonfinishers was greater than the average MSNA response at equal LBNP levels in the four finishers (Fig. 5A):

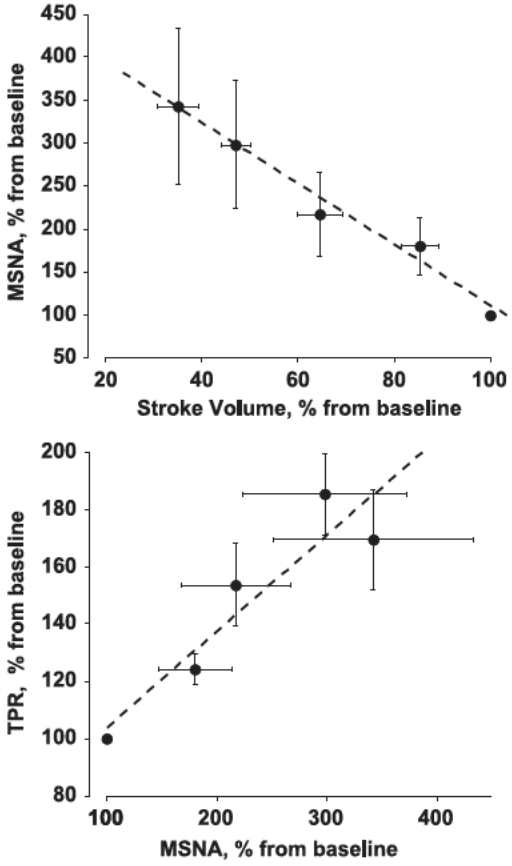

Fig. 4. MSNA is plotted as a function of stroke volume reductions during graded LBNP in panel A, and peripheral vascular resistance is plotted as a function of MSNA during graded LBNP in panel B.

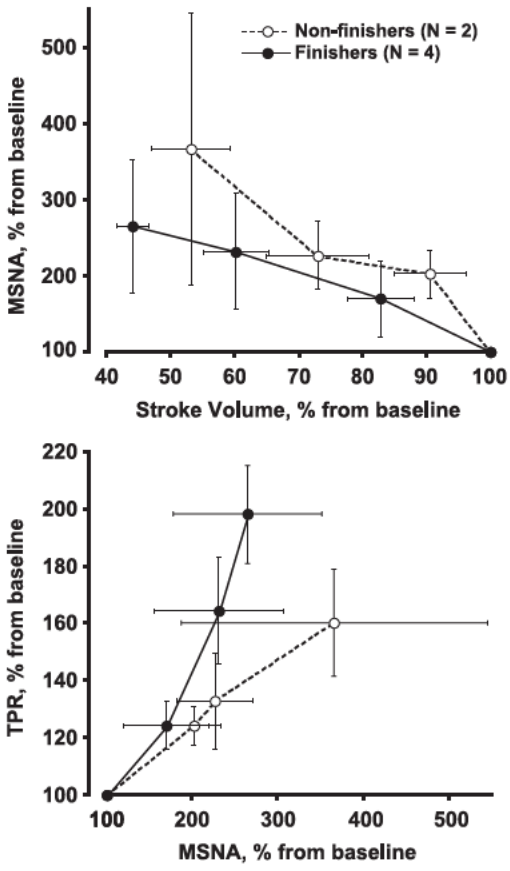

Fig. 5. MSNA recorded from LBNP finishers (closed circles and solid line) and LBNP nonfinishers (open circles and dashed line) is plotted as a function of stroke volume reductions during graded LBNP in panel A, and peripheral vascular resistance of LBNP finishers and LBNP nonfinishers is plotted as a function of MSNA activation in panel B.

despite this, the average peripheral resistance was lower (Fig. 5B).

4. Discussion

We induced graded central hypovolemia using LBNP as a model to simulate the progression of circulatory shock and studied hemodynamic and autonomic responses. Our two primary findings are: (1) MSNA is directly and inversely related to changes in stroke volume; and (2) nonfinishers seem to display relative hyperadrenergic rather than hypoadrenergic function. Contrary to our expectations, our data also suggest that individuals who experience signs of hemodynamic collapse during induced central hypovolemia may have robust sympathetic neural responses but low peripheral vasoconstrictive capacity. We conclude that the development of circulatory shock in at-risk individuals is related more to an abrupt sympathetic withdrawal and perhaps decreased end-organ responsiveness rather than relative hypoadrenergic function.

4.1. Stroke volume–MSNA relations

Central hypovolemia caused by reduced blood volume and/or orthostatic stress leads to lower cardiac filling and stroke volume in accordance with the Frank–Starling relationship. With this construct, the hypothesis has been advanced that reduced cardiac chamber size is neurally transmitted to, and integrated by, the central nervous system, resulting in increased peripheral sympathetic nerve activity (Levine et al., 2002). A proportional elevation in MSNA acts to increase heart rate and peripheral vascular resistance through peripheral vasoconstriction proportionate to the reduction in stroke volume, thus buffering any tendency for development of hypotension (Fig. 4). Although studies examining MSNA responses to progressive central hypovolemia due to trauma or hemorrhage do not exist, clues to understand such relations emerge through examination of the development of hypotension in other groups of apparently healthy people. In this regard, evidence is accumulating from studies on astronauts: 25–65% of astronauts returning from various space missions experience some degree of central hypovolemia resulting in orthostatic hypotension or intolerance (Blomqvist, 1990; Buckey et al., 1996).

Waters et al. (2002) analyzed plasma NE levels as an indicator of sympathetic activation during standing in 35 astronauts following Space Shuttle missions of various durations. Astronauts who presented presyncope during 10 min of active standing had lower plasma NE levels compared to astronauts who tolerated the stand test without incident. The authors concluded that presyncopal astronauts have hypoadrenergic responses and nonpresyncopal astronauts have hyperadrenergic responses to orthostatic stress. However, although plasma NE correlates well with MSNA (Wallin, 1988), plasma NE levels do not reveal peripheral sympathetic responses specifically. Levine et al. (2002) directly recorded MSNA responses in four astronauts during 10-min, 60° head-up tilt before and after a 2-week sojourn to space. In that study, no astronaut experienced signs or symptoms of presyncope, and a tight inverse relation between decreasing stroke volume and increasing MSNA was documented (Levine et al., 2002). However, the data of Levine et al. were generated by only two points (supine and upright tilt) at two different points in time (pre- and post-space flight). Together, these four points were linear, but were not related in time. Our data confirm that the stroke volume–MSNA relation is linear (Fig. 4A), and extend recent observations (Levine et al., 2002) by progressively inducing stroke volume reductions in a graded fashion. With this approach, we provide the first data to verify that in the same subjects during a continuous time interval, reducing central blood volume at five points produces a linear relationship between stroke volume and MSNA. Our results also suggest that the stroke volume–MSNA relation may partially explain mechanisms underlying finishers vs. nonfinishers of LBNP.

4.2. Finishers vs. nonfinishers

Based partly on conclusions drawn by Waters et al. (2002), and partly on anecdotal evidence that survivors of circulatory shock have relatively greater adrenergic stress responses (Orlinsky et al., 2001), we had hypothesized that one mechanism ultimately leading to circulatory shock is a lack of sufficient peripheral sympathetic response to stroke volume reductions (Fig. 1). However, we found that subjects who finished the LBNP protocol without incident increased MSNA less during graded stroke volume reductions than subjects who did not finish the protocol (Fig. 5A). These data indicate it is highly probable that many individuals who experience some form of circulatory shock have equal or greater sympathetic responses than their nonsusceptible counterparts. Lower NE levels measured at the onset of syncope (Waters et al., 2002) may therefore simply reflect abrupt sympathetic neural withdrawal in the face of impending hemodynamic collapse rather than hypoadrenergic function during conditions of significant central hypovolemia. For example, healthy human subjects show well-maintained MSNA up to the point of abrupt hypotension (Cooke and Convertino, 2002; Sanders and Ferguson, 1989; Wallin and Sundlöf, 1982). Several investigators have documented MSNA withdrawal at the point of cardiovascular collapse in both healthy subjects (Sanders and Ferguson, 1989; Wallin and Sundlöf, 1982) and patients with clinical conditions (Morillo et al., 1997; Mosqueda-Garcia et al., 1997). However, subjects in these studies (Morillo et al., 1997; Mosqueda-Garcia et al., 1997; Sanders and Ferguson, 1989; Wallin and Sundlöf, 1982) were not compared to ‘‘normal’’ subjects who did not show signs of circulatory shock, and therefore provide no evidence confirming that these responses were, in fact, hypoadrenergic.

Our data (Cooke and Convertino, 2002) and the data of others (Sanders and Ferguson, 1989; Wallin and Sundlöf, 1982) suggest that sympathetic withdrawal occurs at, or after, the onset of hemodynamic collapse. More importantly, results of the present investigation suggest that LBNP nonfinishers display normal or hyperadrenergic responses to graded stroke volume reductions compared to LBNP finishers (Fig. 5). Therefore, we cannot confirm that susceptibility to hemodynamic collapse is related to hypoadrenergic function. Rather, we support an alternative hypothesis. Development of hypotension becomes imminent at the point when cardiac chronotropic and peripheral vasoconstrictive mechanisms can no longer compensate for progressive reductions in central blood volume. This speculation is supported by the data presented in Fig. 5, showing lower peripheral vascular resistance despite higher MSNA in LBNP nonfinishers. At this point, inadequate cardiac filling can reach a critically low threshold that elicits a vasovagal response consequent to abrupt sympathetic neural withdrawal. We speculate that the underlying mechanism may be revealed, in part, at the level of end-organ responsiveness, whereby adequate efferent sympathetic traffic is not trans-

lated appropriately into increased peripheral vascular resistance. This conclusion is consistent with data suggesting that healthy human subjects who are susceptible to hemodynamic collapse respond to central hypovolemia with smaller elevations in peripheral vascular resistance (Convertino and Sather, 2002; Fritsch-Yelle et al., 1996). However, we also propose that lower vascular resistance may not be caused by inadequate sympathetic activation, as suggested by others (Fritsch-Yelle et al., 1996; Waters et al., 2002).

The duration of each LBNP level (i.e., 12 min) was determined by the time required to obtain physiological data. Consequently, the four levels of LBNP required each subject to undergo a maximal length of LBNP exposure for as much as 48 min. However, the total LBNP exposure time used in the present investigation was not unusually long in comparison to other studies in which subjects have been exposed to LBNP protocols with duration as long as 60–65 min (Levine et al., 1994; Ligtenberg et al., 1998; van Hoeyweghen et al., 2001). Within this construct, it may be of interest to define the relevance of LBNP exposure times to trauma injury. Each LBNP level represents the equivalent of a specific volume of central blood loss (Cooke et al., 2004). In this sense, 12 min is not unusually long. Because of the steady-state, linear relationship of the cardiovascular responses with the magnitude of LBNP, each level of LBNP represents a range of blood volume loss following a hemorrhage in which the bleeding has been controlled. The resulting extended time with central hypovolemia would be analogous to a delay in receiving available fluid resuscitation. This situation certainly represents a likely condition for remote trauma scenarios, especially those experienced on the battlefield.

4.3. Limitations

We acknowledge that the limited MSNA data at higher levels of LBNP hamper our ability to make conclusive interpretations in the absence of a larger sample of subjects that would allow for robust statistical comparisons. In particular, we concede that the second hypothesis (the stroke volume–MSNA relationship will have less slope in subjects who develop symptoms of circulatory shock during progressive LBNP) cannot be adequately supported or refuted with the data presented in this report. It can be extremely difficult to locate the peroneal nerve of certain subjects. Even after the nerve is located, it is necessary to advance the electrode tip into a fascicle such that only efferent traffic is recorded. Nonetheless, we were able to obtain baseline MSNA from 10 of 13 subjects, reflecting a high success rate. Unfortunately, it is a common problem to lose the nerve recording during LBNP. Even without negative pressure applied, any small movement of the electrode (as might occur if the subjects twitch their leg or even move their toes) can cause the tip of the electrode to move beyond the electrode's capacity to record the efferent voltages. Once the LBNP device is turned on, the suction can easily dislodge the electrode and it is quite common to lose the MSNA signal. We have recently published a paper in which this problem is highlighted (Cooke et al., 2004). It should be noted that the difficulties of obtaining MSNA data in human subjects during exposure to severe central hypovolemia is underscored by the absence of such data in the literature (i.e., other than this present study, we are aware of only one other study in which MSNA data at LBNP levels above -50 mm Hg were presented; $n=4$ subjects) (Khan et al., 2002). Thus, our MSNA data at -60 mm Hg, where reductions in central blood volume produce significant hypotension and vasovagal responses in some subjects and not others, are unique.

5. Conclusions

Progressive reductions of stroke volume during LBNP are inversely and linearly related to increases of MSNA. Our hypothesis of blunted MSNA responses to progressive central hypovolemia in nonfinishers cannot be supported. The speculation that nonfinishers display relative hyperadrenergic and not hypoadrenergic responses to LNBP is intriguing but requires confirmation with a larger sample of subjects. Within this constraint, we propose that circulatory collapse subsequent to significant central hypovolemia *causes* withdrawal of sympathetic nerve activity rather than the reverse. Substantiation of our hypothesis with additional data should lead to predictive models for susceptibility of impending circulatory shock in the face of progressive hypotension.

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